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Research Article

Cell-based therapy in thin endometrium syndrome

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Of the known causes of uterine factor infertility, Asherman's syndrome or the so-called intrauterine synechia, chronic endometritis and endometrial hypoplastic processes are most often distinguished. Thin endometrial syndrome is characterized by a decrease in the thickness of the endometrium to 7 mm or less in the proliferative phase of the menstrual cycle and a multiple decrease in the frequency of embryo implantation.

Numerous treatment strategies have so far been proposed for treating refractory thin endometrium syndrome. Recently, cell therapy has been proposed as an ideal alternative for endometrium regeneration, including the employment of stem cells, platelet-rich plasma, and growth factors as therapeutic agents. Single center, prospective, open-label study of efficacy of cell-based therapy in the complex treatment of thin endometrium syndrome in patients with infertility was conducted. The study involved 36 women aged 28 to 36 years, the middle age was 34.2 ± 1.1 years. All patients included in the study received 3 cycles of intrauterine administration of a suspension of autologous stem cells isolated from bone marrow. Bone marrow was successfully aspirated from the iliac crest in all patients. Mononuclear cells was isolated by density gradient centrifugation according to the standard method. The cell material was cryopreservated and thawed immediately before administration. The procedure for intrauterine transplantation of isolated but not cultured cells was performed on the 5^{th}–7^{th} day of the menstrual cycle. There were no significant adverse events related to harvest or administration. The thickness of the endometrium before and after treatment was 2.39 ± 0.64 and 6.56 ± 0.94 mm (t = –21.94, p = 0.0001), respectively. The rate of patients with an endometrial thickness of more than 7 mm after treatment was 77.8%. The effectiveness of assisted reproductive technology in patients with normal endometrial (n = 28) was 32.1%. Immunohistochemistry confirms the presence of chronic endometritis before and after treatment in 22 (61.1%) and 19 (52.8%) patients, respectively, while after treatment a significant decrease in the levels inflammatory markers was found.

Keywords: assisted reproductive technology; bone marrow stem cells; cell-based therapy; chronic endometritis; infertility; regenerative medicine; thin endometrium.

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Научная статья

Клеточная терапия синдрома тонкого эндометрия

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Из известных причин маточного фактора бесплодия наиболее часто выделяют синдром Ашермана или так называемые внутриматочные синехии, хронический эндометрит и гипопластические процессы эндометрия. Синдром тонкого эндометрия характеризуется снижением его толщины до 7 мм и менее в пролиферативную фазу менструального цикла и кратным снижением частоты имплантации эмбриона.

Проведено проспективное открытое исследование по оценке эффективности применения клеточной терапии у пациенток с бесплодием, обусловленным синдромом тонкого эндометрия, и двумя и более неудачными попытками экстракорпорального оплодотворения. В исследование включены 36 пациенток в возрасте от 28 до 36 лет (средний возраст 34,2 ± 1,1 года). Все пациентки получили 3 цикла внутриматочного введения суспензии выделенных аутологичных мультипотентных мезенхимальных стромальных клеток. Для выделения стволовых клеток выполняли забор красного костного мозга в объеме не менее 20 мл из гребня подвздошной кости. Выделение фракции мононуклеарных клеток проводили в градиенте плотности по стандартной методике. Клеточный материал хранился в криовиалах и размораживался непосредственно перед введением. Процедура трансплантации выделенных, но не культивированных клеток проводилась на 5–7-й день менструального цикла.

Толщина эндометрия до и после лечения составила 2,39 ± 0,64 и 6,56 ± 0,94 мм (p = 0,0001) соответственно. Удельный вес пациенток с толщиной эндометрия более 7 мм после лечения составил 77,8 %. Результативность вспомогательных репродуктивных технологий у пациенток с восстановленным клеточной терапией эндометрием (n = 28) составила 32,1 %. Иммуногистохимические данные за наличие хронического эндометрита до и после лечения имели место у 22 (61,1 %) и 19 (52,8 %) пациенток соответственно, при этом после лечения установлено значимое снижение количества провоспалительных маркеров.

Ключевые слова: бесплодие; вспомогательные репродуктивные технологии; клеточная терапия; регенеративная медицина; синдром тонкого эндометрия; стволовые клетки костного мозга; хронический эндометрит.

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BACKGROUND

The number of the population, structure, and reproductive potential are the most important factors that determine the economic development of the country. Currently, the Russian Federation is on the edge of a "demographic abyss," with enormous material and human resources spent on overcoming infertility in infertile marriages [1]. One of the most difficult issues of reproductive medicine, except for male infertility, remains the uterine factor of female infertility, which primarily includes thin endometrium syndrome [2]. Despite the low prevalence of this syndrome, the reduction of endometrial thickness to ⩽7 mm reduces the frequency of embryo implantation by half and in some cases makes pregnancy virtually impossible, leaving only the surrogate option as a fetal receptacle [3]. Except for estrogens, no drugs affect endometrial proliferation [4]. Recombinant vascular endothelial factor, proangiogenic gene therapy, and cell therapy remain the time-tested treatment options [5].

This study aimed to investigate the efficacy of invasive intrauterine injections of uncultured autologous mesenchymal stem cells on endometrial growth in patients with thin endometrium syndrome and repeated implantation failures through in vitro fertilization (IVF) protocols.

MATERIALS AND METHODS

Characteristics of the study sample

The study enrolled 36 patients aged 28–36 (mean age, 34.2 ± 1.1) years with thin endometrium syndrome and two or more failed IVF attempts. For cell therapy, high-quality cryopreserved embryos must be available before the embryo transfer protocol. Before the study, all patients were diagnosed with thin endometrial syndrome by triple M-echo measurement, and all of them had a hypoplastic endometrium confirmed by histological examination of an endometrial biopsy. Estrogen replacement therapy was given at least 6 months, and the condition was not responsive to complex therapy, which included physiotherapeutic treatment and estrogen therapy.

Bone marrow harvesting, isolation, storage, and transplantation of stem cells

After the informed consent form was signed, all women included in the protocol under local anesthesia underwent red bone marrow sampling of at least 20 mL from the iliac crest regardless of the day of the menstrual cycle, following all standard aseptic and antiseptic rules. The mononuclear cell fraction was isolated in a density gradient according to the standard technique. Seeding, expansion, cryopreservation, and reinitiation of cell culture were performed according to standard operating procedures and protocols for manipulating human cells. For reinitiation of culture, multipotent mesenchymal stromal cells (MMSCs) in cryovials were thawed in an aqueous medium to 37°C. The cells were resuspended in the indicated temperature regimen and placed in a CO_2 incubator. When 80% confluence was reached, cells were harvested. The obtained cells were washed three times with physiological solution by centrifugation and removal of the MMSC supernatant at a concentration of 5 mL/mL and packed into 1.0 mL vials under aseptic conditions. At all stages, there was sampling for quality control of isolated cells. Vials with hMSCs were transported in shipping containers for subsequent administration to patients. The MSC culture was administered to them intrauterine [6, 7]. Isolated but not cultured cells were transferred on days 5–7 of the menstrual cycle. A catheter with a 1 mL suspension containing approximately 5 million hMSCs was inserted through a guide into the uterine cavity under echographic navigation. Each patient received three injections of the cell suspension, and ultrasound examination of the pelvic organs was conducted twice during the menstrual cycle, i.e., on days 9–10 and 19–21 of each cycle, to determine the condition of the endometrium.

Endometrial sampling and immunohistochemical study

In one or two menstrual cycles before the start of therapy and on days 19–22 of the 4th menstrual cycle from the start of therapy, endometrial pipelle biopsy was performed with subsequent histological examination and immunohistochemical analysis to determine the expression levels of estrogen receptor (ER) and progesterone receptor (PR) and chronic inflammatory markers. If morphological and echographic endometrial parameters acceptable for endometrial preparation in the unfrozen embryo transfer protocol are obtained, the patient is included in the endometrial preparation protocol for unfrozen embryo transfer into the uterine cavity.

Histological examination was performed according to the classical method using an Olympus CX31 microscope (Japan) at ×100 and ×400 magnifications. Hematoxylin and eosin were used for review staining. Immunohistochemical study was performed according to the standard scheme using antibodies to the ER, PR, CD4, CD8+, CD20+, and CD138+. The results of immunohistochemical reaction were evaluated according to the McCarthy et al. (Hscore) 3-point scale or the specific number of cells (%) expressing the desired antigen. The severity of chronic endometritis was determined according to the generally accepted criteria (Table 1) [7].

Ethical rules and regulations

The study was conducted in full compliance with the ethical principles of scientific and medical research involving human subjects of the Declaration of Helsinki developed by the World Medical Association, current

Table 1. Immunohistochemical criteria of chronic endometritis severity

Detectable antigen	CD8+	CD20+	CD138+	CD4+
Name of the cell subpopulation	Cytotoxic T-lymphocytes	B-lymphocytes	Plasma cells	T-helpers
Evaluated criterion	Number of cells in the field of view			
Norma	Up to 10	Up to 3	0	Up to 10
Weakly pronounced	Increase by a factor of ⩾2		Single cells	Associated with the number of CD8+
Moderately pronounced	Increase by a factor of ⩾3		Increase by 2–3 times or more	
Expressed	Increased by a factor of ⩾5	Increase by a factor of 4–5 or more	Increased by a factor of ⩾5	

Table 2. Indicators of chronic endometritis before and after cell therapy

Indicator	Before treatment *n* (%)	After treatment *n* (%)	χ^2	*p*
Specific weight of chronic endometritis	22 (61.1)	19 (52.8)	0.227	0.635
Changes in the intensity of inflammation despite treatment	–	12 (54.5*)	2.100	<0.001
Degree of severity of chronic endometritis				
Expressed	11 (50.0)	2 (10.5)	5.627	0.018
Moderately pronounced	7 (31.8)	10 (52.6)	1.063	0.303
Weakly pronounced	4 (18.1)	7 (36.8)	0.983	0.322

Note. * of the primary number of patients with CE (*n* = 22).

procedures and standards of medical care, and other applicable regulatory requirements for conducting clinical trials and observational programs in the Russian Federation. The patient observation protocol, treatment, and examination programs were approved by the local ethics committee. Given the absence of the culturing stage of the MMSCs, the study was not subject to the Federal Law of the Russian Federation of June 23, 2016, No. 180-FZ "On Biomedical Cellular Products."

Methods of descriptive statistics used

In the statistical analysis, quantitative features corresponding to the normal distribution were described as mean and standard deviation. Features deviating from the normal distribution were presented as median (indicating confidence interval limits), and qualitative features were presented as fractions (%) of absolute numbers. The distribution of the attributes in the groups was determined by the Harker–Bera test. Comparison of quantitative variable (endometrial thickness before and after treatment) in the groups was performed depending on the normality of distribution, i.e., Student's *t-test* for a normal distribution and Mann–Whitney *U-test* for a non-normal distribution. Frequency correlation for splitting the signs in the groups was conducted using the χ^2 (chi-square) criterion by K. Pearson. In all cases, the critical level of significance was set at $p < 0.05$.

RESULTS

All patients included in the study received three cycles of intracellular injection of a suspension of isolated hMSCs. The mean endometrial M-echo indices before and after MMSC injection were 2.39 ± 0.64 and 6.56 ± 0.94 mm, respectively (Student's *t-test*: $t = -21.94$, $p = 0.0001$). The proportion of patients with an endometrial thickness of ⩾7 mm was 77.8%, and they subsequently underwent a modified natural cycle of unfrozen embryo transfer. In these 28 patients who underwent assisted reproductive technologies, 32.1% (*n* = 9) achieved clinical pregnancy; however, only 21.6% (*n* = 6) had a pregnancy of 12 weeks duration.

No statistically significant differences were found when comparing the expressions of PR and ER in the glandular and stromal components of the endometrium before and after treatment. Immunohistochemical evidence of chronic endometritis before and after treatment was found in 22 (61.1%) and 19 (52.8%) patients, respectively, while the remaining 19 patients showed a diagnostically significant (fivefold) decrease in the number of proinflammatory markers, i.e., cytotoxic T-lymphocytes (CD8+), after treatment, B-lymphocytes (CD20+) and plasma cells in the stromal endometrial component in nine cases (47.4%), and in three cases (15.8%), the expression of proinflammatory markers decreased by threefold (Table 2).

CONCLUSIONS

The application of three injections of isolated but not cultured autologous MMSCs for the treatment of thin endometrium syndrome resulted in a significant increase in its thickness and a decrease in the expression of autoimmune inflammation markers, and it is more effective than all available means of drug and/or complex treatment, in which the response to treatment does not exceed 5%.

ADDITIONAL INFORMATION

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Conflict of Interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

Ethical review. The study was approved by the local ethical committee of the D.O. Ott Research Institute of Obstetrics, Gynecology, and Reproductology (Protocol no. 11/10 of 11.10.2021).

Authors' Contributions. All authors contributed substantially to the study and the article, and read and approved the final version before publication.

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